As a result of recent advances in cell biology and the rapid progress of the human genome project, we have arrived at the starting point in the race to understand how cells and organs really work. The problems are immensely difficult, but the potential benefits are extraordinarily great for those who seek to understand biology or to help the disabled.

So, how will stem cells do this? First, we know that they will serve as a direct source for cells in grafts to function in those organs which are diseased or damaged. Secondly, the understanding of what a stem cell is from the standpoint of its molecular signature and how it goes about specializing into specific cell types will enable us eventually to use this information to directly convert cells in the body to stem cells and then to have those cells specialize into the tissue that is needed.

Obviously, the scientific challenges to attain our goal are formidable, and it is going to take the work of large interdisciplinary groups from the lab bench to the clinic to make this work.

Now, I want to address most of my comments today on where stem cell biology is with respect to the cardiovascular system. One point that I want to make up front with respect to stem cells in general and their potential is illustrated in this slide, which is a bit dated, but still it shows, I think, from the public side, this desire not only to have such technology, but also, and a dangerous one, that is probably already here. And this is something which I will address at the end of the talk with respect to circumspection in this area of research.

Well, our first goal is to discuss what stem cells are. Even among stem cell biologists, there are differences of opinion, but for the sake of our discussion on this topic, we will say that a stem cell has to have two properties. The first property is that of self renewal, which means that it is capable of generating more cells like itself. The second property is that a stem cell is capable of specializing into some cell type. It can be many different cell types, or it can be a single cell type. For example, the stem cells in the skin will only form one cell type, whereas some of the cells we will be talking about today can form many different ones. So, those two properties are essential for a stem cell.

We also use other terms in stem cell biology such as a progenitor cell, or a transit amplifying cell, or a differentiating cell. And there is confusion but, again, for the sake of our topic today, as long as it is understood that a stem cell is a cell that has the two properties we just talked about, as differentiation begins in a lineage, we then form what is called a progenitor cell, or a transit amplifying cell. Now this cell still has the ability to produce cells like itself, but it is more committed in the specialization pathway, such that you now have, for example, neural stem cells, or muscle stem cells, connoting really the fact that it is now limited as to what its potential can be. And the differentiated cell is one, then, which has full characteristics of any specific cell type that we are talking about in the body.

Where do stem cells come from? And in this diagram, we illustrate those that come from embryonic, fetal and adult sources. From the pre-implantation staged embryos, it is possible to generate a stem cell which is called an embryonic stem cell. From the fetal stages, we can generate a number of different stem cells. Some of them are referred to as embryonic germ cells, since we generate them from the actual developing germ line tissues, or we can generate stem cells from various organs and tissues, be they neural stem cells, muscle stem cells, etc.

From the adult, we can generate a variety of stem cells, and this, over the past few years, has received a lot more attention, probably driven more by the fact that there is controversy with the embryonic and fetal sources.

Well, what are some of the stem cells that we can isolate from adult tissues? Well, we've known for decades about hematopoietic stem cells, those cells that can give rise to the blood lineages. Mesenchymal stem cells were first isolated in the 1970's. The sources of these come from bone marrow from a number of different solid organs. Neural stem cells, this is another hot topic that really got going but no more than five to seven years ago when it was recognized that, for example, within the adult nervous system, that there were actually stem cell populations, but you had to remove the cells from the CNS, and place them in culture before you recognized that they were actually stem cells, which could then be grafted back, and they would differentiate appropriately in the CNS.

The multipotent adult progenitor cell, which is very closely related to the mesenchymal stem cell, is a source that Katherine Refy has made famous. It is a cell type that has different growth parameters than mesenchymal but can effectively do the same thing.

Quite recently, umbilical cord blood has served as a source for stem cells, and it has developed a small industry in saving umbilical cords and generating stem cells from that blood. The decidual teeth of young children have stem cells in them called shed cells, so now, instead of placing your child's tooth under a pillow, you should extract the cells and freeze them down and save them for potential use in the future.

And finally, over the last year, there has been a great deal of excitement, and relevant to what we are going to talk about, is the realization that the myocardium in the heart does house stem cells, or progenitor cells, and two groups recently have reported those, and we will talk about them later in this discussion.

There is a great deal of controversy in the stem cell field, and it centers around really two areas. One is what criteria we have for a stem cell, such that when someone isolates a cell type and then uses it in some type of transplantation paradigm, that we give appropriate credit to what was responsible for the outcome of those grafts. And so, there is a great deal of effort in our field currently to establish criteria that we all agree upon to define a stem cell.

Secondly, there has been a great deal of controversy in the issue of reproducibility of results. And, in my career in science, I have never seen so many papers published in leading journals — Nature, Cell, Science — in which people are producing negative results as a result of prior publications producing positive results, and then those results are challenged and subsequently do not hold up with respect to reproducibility. And so, it is kind of disconcerting in a way to see this, but it is also important. As you can appreciate in science, results must be reproducible.

So this has led, in time, to a great deal of concern about the interpretation of the results that we are seeing and the claims and, unfortunately, and this is the only political comment I'll make in this talk, is that part of this is being driven by concern in some quarters over the use of the embryonic and fetal sources of stem cells. And so, much more is then touted about, for example, certain types of adult stem cells. And so we are concerned about how this political issue impacts on the science of stem cell biology. But it is real, and we must be very much aware in treating publications as scientific ones and addressing the issues of science.

Well, let us get into some of the biology of this and some of the real problems in the system. We are going to be spending a bit of time talking about embryonic stem cell sources, but it is not unlike other stem cell sources in that we have one major issue, and the greatest obstacle at this point in time in this field is the following: that if in a dish, we have a stem cell population, we are faced then with figuring out how we get that stem cell to produce the cell type we want with high efficiency and to the end point of producing a fully differentiated cell.

So, if we take an embryonic stem cell, which we know is capable of producing all 200 cell types in the body, how do we instruct, how do we guide it, how do we enhance it, if we only want liver cells, or podocytes , or adrenergic neurons, or pancreatic islet cells, or cardiomyocytes. And this is where the thrust of research is today. How do we get these cells to do what we want? That is basically it. How do we instruct them?

We rely heavily on what our colleagues have found in molecular embryology that is important as far as ligands and factors that are involved in the differentiation of cells into different lineages. But still, the state of the art is that, in a dish, at this point, even under the best of circumstances, you will get a mixture of cell types produced. There may be predominantly the one you want, but it is still a mixture. And clearly, this is not good enough for grafting, principally because of safety reasons. So, we have to rely upon selective strategies to pull out specifically the cell types we want. So we can use selectable genetic markers, we can use specific types of growth media, or we can sort cells based on what is present on their surface, and sometimes what's present in their cytoplasm. So, that is the state of the art. We can then pull out, or enrich further, the populations of cells that are differentiating from the stem cells in a dish.

And then ultimately, in our paradigms, we will take these individual cell populations or, now that we are working more closely with the biomedical engineers, we will combine them to form tissues or organoids to graft into, at this point, laboratory animals. So that, all stem

cell biology has these issues in common. And I just want to point out a few critical ones that, again, apply to all areas of stem cell biology. This is absolutely relevant to the cardiomyocyte differentiation that we are going to talk about.

Well, we are clearly interested in what we mean by stemness. Is there a molecular definition of this, and there is a lot of work being done at the genomic and proteomic levels and chromatin structural levels to try to understand what it is within a stem cell that makes it a stem cell. Because, ultimately, we would like to take any cell in the human body and convert it to a stem cell. And this becomes particularly important when we want to get around the immunologic barriers of tissue grafting which we will mention in a moment.

We need these high efficiency differentiation protocols. As you will learn when we talk about the cardiomyocytes, even under the best of conditions now in culture, we can only generate less than 5 percent of a cell population of cardiomyocytes. Other cells we can get maybe 80 percent, so that becomes an issue, and we do this by controlling the environment and by genetic manipulations.

We are concerned that the cell type that is derived in a dish is authentic, which means it *is* the cell type that you have in your body. And there is a great deal of effort being made at this point to assess that authenticity in many ways, and we will talk about that. We are interested in contrasting and comparing among various sources of stem cells. And then the whole issue of quality control, with cells in culture, if they can divide endlessly, etc., were considered about genetic changes as well.

In testing these cells, in animal paradigms, and in getting prepared for human studies, there are also issues which impact on our understanding, or interpretations, following grafting. First, do we know enough about the disease and injury that we are trying to ameliorate? And, of course, one could argue that we really don't and, therefore, we shouldn't go forward, and some people have put forward that argument. But, clearly, we have to understand this to the best of our knowledge so that we can interpret the results. What cell types should we be putting in, where should we be putting them, and what outcome should we expect from those grafts?

Are there appropriate animal models available in which to test the cells? And this is a bone of major contention in the stem cell field. And then, what stage of cell differentiation do you graft? Do you take the very, very early stage, for example, progenitor cells, or do you take something much later? This we must determine empirically. How do you deliver those cells is another issue, and that is obviously organ and tissue specific. Safety issues — what happens to the cells that are grafted? Do they migrate away, do they differentiate appropriately, do they form tumors — all considerations that are of great interest.

And then, finally, the issue of the immune response. How do we get around it other than taking cells from the individual that you're going to graft back to? We have a lot of work

ahead of us with respect to trying to induce tolerance, genetically altering cells, etc. These are major, major issues in all of stem cell-based interventions.

Well, let us talk then more specifically about cardiomyocytes and where the field is in utilizing stem cell biology for producing cardiomyocytes that could be used in cell-based therapies. One recognizes the importance of heart disease, heart failure, injury, and clearly it is an area in which we have the capacity to begin looking at stem cell therapies, and this is obviously what is driving the field. If you look at the top areas of stem cell work at this point, one finds at the top of this list diabetes, cardiomyogenesis areas, stroke, Parkinson's disease, really the top kinds of disease, spinal cord injury, the top things we are faced with. So, this is what's really driving this field in many ways at this point.

So, what therapeutic strategies does one have currently? Well, one can replace the heart or, perhaps, one can now begin to consider cellular transplantation for specific populations of cells within the heart. I think it is fair to say that our expectations would be that, with the cellular transplantation, that we can produce functional cardiomyocytes, that we could perhaps inhibit scar tissue formation, and then see ultimately an improvement in heart function and relief from the symptoms of heart failure.

Now, this is against the background, which most of you appreciate, that terminally differentiated cardiomyocytes are unable to enter the cell cycle and divide. And so this doesn't make it unique among specialized types, but it seems to be a property that is present in the cells at the time you understand that they're going to become a cardiomyocyte very, very early in development. This is rather unusual. It doesn't seem as if it has an amplifying stage at all to it. Now, given this, it has made it a little bit more difficult in many laboratories to produce large numbers of cardiomyocytes from any stem cell source.

Now the issue of whether or not the heart, itself, contains a stem cell population seemed to have been resolved years ago when there was no evidence of any cell division beyond birth of any cell type within the heart, any cardiomyocyte cell type, but now there are recent reports, and we will come back to that, that challenged this dogma, so there may be another way around the goal to get cardiomyocytes, and we will talk about that in a few minutes.

Now, let us say that you wanted an ideal cardiac stem or progenitor cell. What would be the characteristics of this cell? And I think it is important that we go through some of these requirements so that you understand the necessity of, and vigilance of, making sure that the cell type that you want has all the properties you want, which means it is an authentic cardiomyocyte.

So, what are some of these? Well, clearly, you want the cell to be able to differentiate into a fully mature cardiomyocyte and, we would say, you know, in a dish, if possible. Not an absolute requirement, but it has to be a fully mature, authentic cardiomyocyte. You want that cell to function in vivo, in either embryonic or post-natal hearts, and that that cell could be

localized to a specific region of the heart and assume the parameters of the cell types in that region. We would hope that we could identify the specific molecular cues that would drive the differentiation of that cell into this fully mature cardiac muscle cell. That way we could reproducibly, in a dish, generate the large numbers of cells that would be required in any type of graft intervention.

We would like to use markers of some kinds within those cells that we could recover the cells, at least in the experimental part of this, from the graft and show that, indeed, they have the properties that we want of an authentic cell. And, clearly, an outcome here has to be improvement of organ function as a direct result of the differentiation of that stem cell. We would also hope that that differentiated function would be stable and it would have s long-term presence, which means we don't want to continually graft cells, or that they would not be lost for other reasons.

And finally, we are getting these wonderful results in rodents in stem cell work, but we have to be able to extrapolate this to larger animals. And in the case of heart work, where you do have difference in heart rates and physiology, that we want to do it in animals that better reflect the human cardiac physiology, and that is going to be a challenge.

So, where is a jumping off point for some of this work in the use of any type of a stem cell, or early lineage cell, for grafting of studies? This is an example from Loren Field's work in the 1990's in which he was able to demonstrate that he could take fetal cardiomyocytes and graft them into the myocardium of the mouse. And this illustrates nicely through the use of a cell marker, which is in blue here, showing the cells that were grafted to the heart, and they appear to function. But these are fetal myocardiocytes. Is this something that could be done in humans? Absolutely. But what one thinks of what it would take from the standpoint of the collection of fetal tissue, it is a procedure that probably would not be endorsed, and certainly we would not have enough material to use in the clinic. But it illustrates that these cells in an early stage lineage can be grafted and can function.

Now, with the development of the mouse embryonic stem cell system, which began in the 1980's, there were investigators looking at the differentiation of these cells, in culture, into a variety of cell types. Again, led by Loren Field and Anna Wobus , cardiomyocytes were recovered from embryonic stem cell cultures. In a spontaneous fashion, they represented a very small portion of any differentiated cell type in the dish. And so, it was necessary, as illustrated in this slide, that if you use genetic constructs that would rely on the promoter regions of genes that are expressed early in cardiomyogenesis, that it would be possible not only to recover the cardiomyogenic cells, but to do it with a high degree of purity, in this case, 99.6 percent of the cells that are recovered using a gene construct in which, downstream of it, you have a selectable marker that it could be used efficiently. And this has become the standard in the field, both in fusing to an antibiotic resistance or in some type of selection with a marker that would light up that one could then use fluorescence to select for.

This has even taken a step further, where specific sub-types of ventricular cells can be isolated out of embryonic stem cell cultures, again by the choice of the promoter of the gene

that would characterize that sub-type and taking the promoter of that gene and coupling it to a reporter system, or a selectable system, as is in the case illustrated in this particular study here from Anna Wobus .

Now, a number of labs have worked on mouse ES derived cardiomyocytes, and what have we learned through this? Well, as I mentioned, the average content of these cultures, even under the best conditions, would represent less than five percent of the cells being cardiomyocytes. And so, it meant you had to start with an inordinately high number of cells, embryonic stem cells, to recover any significant number of cardiomyocytes. So, clearly, the efficiency of differentiating these cells has to be improved.

They're against the background of many other cell types, and so if we have to use an enrichment, or selection method, to pull these cells out. Then, once these cells are out, investigators are working on efficient delivery methods to get these cells back into the myocardium and to minimize cell death upon those transplants. And this has proved also to be somewhat difficult.

And finally, if we want to think more in the longer term, whereas you can easily use immunosuppressive therapies in the mice, we are thinking of the alternatives of using therapeutic cloning or tolerance induction. So, it can be done; embryonic stem cells can be a source of myocardiocytes, but with very low frequency.

More recently, Lior Gepstein, in Israel, who's been the leader in working with human embryonic stem cells and generating cardiomyocytes, has published several papers illustrating that you can recover cardiomyocytes, that they have the properties of cardiomyocytes. He's done quite a bit of electrophysiology on these cells. But, again, the frequency of finding these cells in culture is extremely low.

The other revelation that has come out of this, and looking back at the mouse work as well, is that the myocardiocytes that are recovered represent, or have a fetal phenotype to them. They do not have the properties of fully mature cardiomyocytes. So, it is not clear what this means, whether we are missing something in this differentiation process in the culture dish; can we genetically enhance this in some way or, clearly, this is a focus at this point in time in trying to derive myocardiocytes from embryonic stem cells.

So, in summary from this work, the ES cells are serving as a source of cardiomyocytes. They display structural and functional properties of early stage cardiomyocytes. We are hopeful that we will be able to overcome those problems of driving a fully mature cardiomyocyte with much higher frequency. So, those are some of the issues from that stem cell source at this point in time.

So, we are going to switch gears, and we are going to look at some other sources of stem cells that have been used to derive myocardiocytes. And, for a period of time, the most exciting source turned out to be hematopoietic stem cells. Now these are cells, as you know,

that are capable of reconstituting and maintaining the blood cell lineages and endothelial tissues. Hematopoietic stem cells are usually defined based on cell surface markers such as CD34, etc., etc., etc., and that is how they are identified, and that is how they are recovered from bone marrow, for example. You can also use those markers to purify populations of the cells so that you could only deal with hematopoietic stem cells.

Now, there were a number of papers published in 2000, 2001, and 2002 that had a very unusual and very exciting result, and the results were so exciting in this area with respect to the contribution of these stem cells to the heart that clinical trials were actually initiated using hematopoietic stem cells as the source of cells for cardiac damage. And so, what were those studies? What did these studies actually say? And that is that hematopoietic stem cells, when introduced into animal models, for example, of ischemic cardiac muscle, that these animals showed evidence that the cells were integrated into the heart, and they showed evidence of functional improvement of the heart.

And there were a series of these, as illustrated over the next several slides, from Don Orlic's group, from Peg Udell's group, and even some clinical papers are included here to indicate that, yes, derivatives of these cells were found in the heart following grafts, and there was an improvement in cardiac function. And so, this was very exciting news. Most of these cells were delivered IV, so there was a homing to the heart to the site of injury which said, gee, this may be a nice way of introducing the cells, delivering the cells to patients.

One example of those experiments from Peg Udell's group is illustrated in this slide in which a mouse is radiated, a coronary artery ligated, and then a population of cells that she refers to as a side population following facs sorting, was introduced IV into these animals and, as you can see from this table, there was a contribution not only to the cardiomyocyte compartment, very low, .02 percent, but there was also a nice contribution to the endothelial cells within the heart, at 3.3 percent, and these animals are showed recovery.

With Don Orlic's group, the results were even more exciting in which, again, a mouse with a damaged heart received an injection of hematopoietic stem cells and is illustrated in some of the immunocytochemistry shown here that the cells in green represented the donor cells, that there was a great contribution of those donor cells to the myocardial regions of the heart. In fact, 54 percent of the myocytes seen in these illustrations came from the donor cells. It was this type of data, combined with another procedure that was used in which an animal, before receiving a cardiac lesion, was treated with several factors, or two factors actually; one was stem cell factor, and another was granulocyte colony stimulating factor injected into the animals, and then the arteries were ligated, and it was seen that these animals recovered very rapidly and had even a higher percentage of cells, as illustrated in this slide, within their myocardium coming from the mobilized bone marrow cells within the animal. Seventy percent of the cells found in the heart were from immobilized cells.

This was carried out in a clinical trial, in the first clinical trial; illustrated in this case. Here, we are giving the GMS CSF, and you can see from the outcome, at least with respect to

the collateral flow that there is an increase in function in these patients. So this was extremely exciting information to say, gosh, you could combine hematopoietic stem cells and mobilize them, for example, that this would lead to contributions to the heart. So, when this came out, as I mentioned, in the early 2000's, we were excited by the results.

So, what was going on here, apparently, was that circulating cells could find their way, with some kind of chemo attraction, to the damaged heart and could populate the cardiomyocyte and endothelial lineages within the heart. Well, it turns out that wasn't the case. And there were two major concerns about these studies as time went on.

The first was the revelation, and this was a major revelation in stem cell biology that many cells, when introduced into an animal, will fuse with the animal's own cells. In other words, two cells, you have a donor cell and a host cell, and it could be in the central nervous system, in the spinal cord, anywhere, liver, that a donor cell coming in would fuse with a host cell. And this, then, raised the issue of, well, what happens in this hybrid cell? Does it take on the features of the cell type you want, or that you're needing to replace?

We still don't know some of the answers to this but, clearly, this explains why we find some of the label from the donor cells in a specific cell type, because it is fused with that cell type in the animal. And we can certainly make use of this, I think, ultimately as a therapy but, in most cases, it gives you a false result as to what that cell you put in is capable of making. It is only fused with the cell, a specific cell, within the animal.

And we now have many examples of this. This is a cartoon illustrating that process in which we can find high frequencies of fusion in the central nervous system, in the liver, in the spinal cord of donor cells with host cells. So, some of this was at the basis of the results that were seen in the early studies.

But, most importantly, we find that the conducting, the conductance, of those studies used poor experimental strategy. Recently, two papers have been published in Nature, and they're referenced here in which it has been unequivocally shown, unequivocally, by the use of genetic markers and extensive confocal microscopic studies of these tissues that the hematopoietic stem cells do not differentiate into cardiac myocytes in these damaged hearts. And I can't emphasize this enough. I would urge you to read these papers; they are just extremely well-done studies.

So, what was the difference here? Why in these studies did they not see this? Why was it so heavily reported in these other and the earlier studies? On this slide are the two strategies that we used in the two studies in which genetically marked cells were used rather than relying upon immunohistochemical differences between human cells and mouse cells, which was done in the early studies. Here we had genetic markers, and we had genetic markers in which you could also detect the cell fusions if this is what you were looking for versus the free-standing grafted cells.

So, yes indeed, as shown on the right-hand side of this panel, you could find donor cells within the heart. In both of these studies, they found it but in no case did they find any of the hematopoietic stem cells forming myocytes. Yes, they found contributions to the endothelium, no question about that, but none of these, and not once did they see a myocyte from the donor's tissue. So, I think this is a major contribution and one that we should be very careful of in the future of jumping in very quickly to utilize an experimental strategy that was not completely well thought out.

Now, it is clear that there was improvement in the function, for example, the output of the hearts in these animals and in patients that have received their own hematopoietic stem cells. And one could argue that this is sufficient to use this procedure when we know that what these cells are actually doing now, the hematopoietic cells, they're behaving as hematopoietic cells, they are honing to the heart, and they are forming blood vessels, microcapillaries on the surface of the heart which, obviously, is helping that damaged muscle perform. So, the extent of this remains to be seen, but that is the sole mechanism whereby these hearts are having improved outcome, not through the integration of new cardiomyocytes within the heart.

Another source of stem cells that have been used to generate cardiomyocytes come from mesenchymal , or referred to as mesenchymal stem cells, and these cells can be derived, or isolated, from bone marrow, muscle, skin, adipose tissue, etc. They have a certain collection of cell surface markers that are used and have been shown and demonstrated nicely in culture to develop into cardiomyocytes when treated, for example, with 5-azacytidine . So, the mesenchymal stem cells are proving to be a source. Again, the issue, though, is how effective will they ultimately be?

This is an example from the Toma et al study showing, and this is an important illustration because on these panels on the right, it is the fluorescinated, the yellow green, that represents the contribution, and I think you can see even in the selected field that those contributions are very low to the cardiomyocyte pool within the heart.

Now, an interesting use of these mesenchymal stem cells is reported in this paper by Mangi et al in which the mesenchymal stem cells were transfected with a virus that contained a pro-survival factor, and these cells were then introduced into an animal that had an infarct lesion, and you can see this contribution to the infarct lesion under the second set of panels, they're in green there, and it resulted, as illustrated in the table, not only in normalized systolic and diastolic function of the heart, but also in a vast reduction in the volume of the infarcted area as shown in the last part of that bar graph. Very impressive study, and clearly this type of approach could be utilized once safety things are concerned downstream. So, this is a nice study of mesenchymal stem cells in an animal model of an infarct.

Now, the last topic I want to mention, and probably the most exciting, that has occurred in the field of stem cells and the heart has been the recent report from two groups on the isolation, the identification and isolation of cardiac stem cells, or cardiac progenitor cells,

within the adult myocardium. And, obviously, this is extremely important. If, in fact, our hearts do contain these populations of stem cells, we now can ask the questions of whether they can be isolated efficiently, whether we can then utilize them in any kind of cell-based therapy. And so, let us spend a few minutes on what these cells are about.

So, in this panel here, from the Texas group, it shows the isolation of the cardiac stem cells using a cell surface marker, Sca-1. In the top three panels when, if you deplete the myocyte population of this flow sorted group of cells, and these are dissociated heart cells, if you remove the myocytes, 14 to 15 percent of the cells you recover are these cells with the Sca-1marker, and it turns out that, although they do have Sca-1, which is in common with bone marrow stem cells, that they don't have any of the other markers of any of the blood-derived stem cells. So they do represent a unique group of cells. Isolated with Sca-1, you can also throw in C-kit and a few other markers, negative markers, to isolate these cells.

And they went on to show that when you take these cells and graft them back into an animal that has an ischemic heart injury and using, again, a series, a nice clever use of genetic markers, that they can show that these cells will form cardiomyocytes, half of which will fuse with the host cardiomyocytes, and they could do this again distinguishing which of the donor cells were and whether they are individual or fused, but they can show that, indeed, they will form cardiomyocytes, and those cardiomyocytes are functional, which is a very impressive study. So, they're not only able to isolate these cells, to have some form of characterization, they can graft them back in and show that they will form cardiomyocytes.

The second group, and I'll summarize their information here, used Lin minus C-kit plus cells, flow sorted for those and found a large number of self-renewing, clonogenic, and multipotent cells in this population and that they could inject these cells into hearts and show that, again, they will reconstitute myocardial and blood vessels as well. So, it is a very impressive beginning, I think. These papers were just published at the end of last year, and we are now hearing at conferences more data coming out about the fact that the heart has these stem cells.

Now, how important is this going to be? Well, we can't answer how important it is going to be because, you know, now we've known for a number of years that the CNS has stem cells, but how we could mobilize them, how we could use them, in any type of therapeutic way still remains a problem. And so one would argue well, how do we mobilize these stem cells? How can we, you know, if they're there, why don't they help in heart disease, or heart injury? Still, we have a number of enigmatic questions to answer at this point in time, but the fact of the matter is that the heart appears to contain a population of stem cells.

So, how do we summarize, what can we summarize here as far as what we are seeing. Well, we are seeing that there appears to be several sources of stem cells for cardiomyocytes in different degrees of development, so to speak. Whether they're embryonic stem cells, whether they are mesenchymal stem cells, or whether they represent cardiac stem cells right

out of the heart itself. Based on this, obviously one has to be optimistic that the future of cardiac stem cell therapy is bright but, as in the other areas of stem cell biology at this point in time, it is going to take a concerted effort and a lot of resources invested in this work to determine, really, which of these is going to be the best, which ones are going to differentiate appropriately under controlled conditions, can these cells be expanded to the numbers that one would need, can they be grafted efficiently, and will they possess the properties of authentic fully differentiated cardiomyocytes.

I is easy to say what our demands will be, and it is certainly going to take a while, I think, for any of these potential sources to really develop, I believe, the types of cells one would need to do this efficiently and safely.

Let us go beyond where we are for a moment and, to make some predictions. I certainly feel that we will come up with cell-based therapies for the heart from one or several of these sources. I think also, though, learning what we can about stem cells, about stem cells that will differentiate to form cardiomyocytes, but the information that we are going to gain in these studies is going to ultimately be more important than the stem cells we derive, or the differentiation protocols that we establish. And that information that we gain on how to do this, I think, will ultimately be used on a patient's own cells, such that the clinic of the future would be one in which, if there is stem cell therapy required, that the patient's own cells will be taken, converted to stem cells, and then utilized with existing protocols to develop cardiomyocytes, or whatever other cell type would be needed within the heart for cell-based therapy.

I think we are seeing already evidence of this in some interesting experiments, not with the cardiomyocytes, but with other cell types. We are beginning to learn how to do some of these things in a dish, just taking fibroblast as starting material.

To me, there is also a word of caution. The stem cell field is moving extremely fast, rapidly. We are getting a lot of interesting results, some of which we are having a difficult time interpreting. And I have given you one example where the interpretation was completely wrong. And I think we have to be somewhat circumspect in our desire to rapidly move this information into the clinic. I am concerned about safety issues which, I think, could really defeat our goals here if we move too quickly. And again, I think, to be able to provide a cell-based therapy for damaged hearts that will be efficient and will be safe is still going to be several years away at the earliest. But I am optimistic. I think it is one of the areas that has some of the highest visibility and clearly one of the highest needs.